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CHARACTERIZATION OF COMPONENTS OF P-700-CHLOROPHYLL *a*-PROTEIN COMPLEX FROM A BLUE-GREEN ALGA, *PHORMIDIUM LURIDUM*

CINNIA HUANG, DONALD S. BERNS * and DON U. GUARINO

Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201 (U.S.A.)

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The P-700-chlorophyll *a*-protein complex (F60) isolated previously by a nondetergent method (Huang, C. and Berns, D.S. (1983) Arch. Biochem. Biophys. 220, 145–154) has been treated with the anionic detergent sodium dodecyl sulfate (SDS), and the two resulting fractions, designated (for heavy) H and (for light) L, were separated by chromatography on a Sephacryl S-200 column. After removing detergent, the isolated H-fraction contains about 43 chlorophylls per P-700 molecule. At −196°C, the fluorescence emission peak of the H-fraction is at 715 nm; in the presence of detergent a major 677 nm and a minor 720 nm maximum are observed. The low-temperature emission maximum of the L-fraction is shifted from 686 to 677 nm in the presence of detergent. Two bands at approx. 696 and 654 nm are observed in the chemically induced difference spectra of F60 and the H-fraction in SDS-Tris buffer, but principally one band at 654 nm is observed in the L-fraction. After exhaustive dialysis in Tris buffer, the major 696 nm band is dominant in all samples and there is a great attenuation of the 654 nm band. The polypeptide composition of F60 and the H-fraction are similar while the L-band consists of polypeptides lower than 20 kDa. The amino acid composition of F60 and its two components (H and L) is also reported. Sedimentation-velocity studies indicate that in SDS-Tris buffer the H-fraction contains two species, 2.5 and 10.1 S, and in Tris buffer one species, 14.7 S, while the L-fraction contains one species, 2.5 S, with or without the detergent. The results presented here suggest that the heart of Photosystem I is mainly localized in the H-fraction and that the light-harvesting chlorophyll-protein complex is in the L-fraction.

Introduction

Ogawa et al. [1] and Thornber et al. [2] were the first to demonstrate that Chl is associated with proteins to form discrete Chl-protein complexes. To identify and characterize these complexes, the common approach is to solubilize the chloroplast membrane with detergents and then separate the solubilized material by SDS-polyacrylamide gel electrophoresis. Two Chl-protein complexes were previously resolved by SDS-polyacrylamide gel electrophoresis [1,2]: CP I, which represents the core of PS I, P-700 [3,4], and CP II, which is believed to function in harvesting light energy for both photosystems [3–5]. Isolated CP I contains about 40 antenna Chl per P-700 molecule [6–8]. Bengis and Nelson [9] have reported greater P-700 enrichment (10–20 Chl/P-700) by using Triton X-100 and chromatographic procedures. A mixture of Triton/LDAO/SDS was used by Alberte and Thornber [10] to isolate the PS I reaction center in a highly enriched form (15–25 Chl/P-700). Newman and Sherman [11] have shown that

* To whom correspondence should be addressed.

Abbreviations: Chl, chlorophyll; LDAO, lauryldimethylamine *N*-oxide; PS I, II, Photosystem I, II; Me_2SO, dimethyl sulfoxide; CF I, II, chlorophyll-protein complex I, II.

the PS I particle from a blue-green alga contains five polypeptides (61, 18, 17, 15 and 14 kDa). Other investigators [12–14,32] have reported that CP I from either algae or higher plants contains a single polypeptide chain (64–66 or 58–62 kDa) and that CP II contains one to six polypeptides. The discrepancy in the polypeptide compositions may result from the various detergents used in the isolation procedures. Shiozawa et al. [15] indicated loss of the P-700 activity and spectral alteration of Chl *a* by SDS treatment. Furthermore, the ratios of Chl *a* to P-700 in the P-700-Chl *a*-protein complex (F60) were affected by the types of detergent used [16].

In the past, attention has been focused on the isolation of the reaction center by using various detergents. Little is understood concerning its structure in the complete PS I system. We have recently developed a procedure [17,18] to isolate F60 from a blue-green alga, *Phormidium luridum*, in the absence of detergents. The isolated complex is suggested to be closer to the native state of PS I in vivo. By this procedure, using F60 as a starting material, we were able to separate two components by a chromatographic procedure in the presence of detergent. Their polypeptide and amino acid compositions, as well as the results of sedimentation-velocity studies, are reported here.

Experimental procedures

Preparations. F60 was isolated from a laboratory culture of a blue-green alga, *P. luridum*. The complex was extracted and purified at pH 8.0 in Tris buffer (50 mM) in the absence of detergent, as described previously [17,18]. The anionic detergent SDS was then added to F60 (SDS/Chl $a = 6:1$, w/w). The SDS-treated complex was passed through a Sephacryl S-200 column (2.7×20 cm) and eluted with 0.05% SDS-Tris buffer at pH 8.0. Two fractions were collected and dialyzed in Tris buffer with several changes at 4°C.

Analytical methods. Absorption spectra, fluorescence emission spectra, and P-700 activity were measured as described previously [17,18]. The samples were extracted with 80% acetone, and the Chl *a* concentrations were determined by using an extinction coefficient of $7.54 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ at 663 nm [19].

Electrophoresis was performed in a slab gel apparatus according to Laemmli [20]. The analyzing gel contained 7.5% acrylamide for unheated and 12% for heated samples. In each case, 3% acrylamide was used for the stacking gel. Unheated samples were treated with 1.25% SDS and freshly prepared before the run. Heated samples were treated with 1.25% SDS and 1.25% β-mercaptoethanol and boiled for 2 min. The gel was run about 5 h at a constant current of 30 mA, then stained overnight in methanol/acetic acid/water (40 : 10 : 50, v/v) containing 0.1% Coomassie brilliant blue and destained in methanol/acetic acid/water (40 : 7.5 : 42.5, v/v).

Amino acid compositions were determined as follows. Protein samples were hydrolyzed in sealed, evacuated vials containing 0.5 ml of constant-boiling 6 M HCl for 24, 48 or 72 h at 110°C. Hydrolysis of samples for the purpose of cysteine determination was essentially the same, except that 5 μl of Me_2SO was included in the vial and hydrolysis time was 21 h [21]. After hydrolysis the vial contents were evaporated under reduced pressure to dryness. The residue was redissolved in 0.2 M sodium citrate buffer (pH 3.2) for chromatography. Chromatography was performed on a Beckman 119 CL amino-acid analyzer with utilizing the Beckman single-column hydrolysate system.

Sedimentation velocities were determined in the Spinco Model E ultracentrifuge with absorption optics. Data were collected by photoelectric scanner and recorded with the monochromator in the blue-light region.

Results

Absorption and fluorescence spectra

The elution pattern from a Sephacryl S-200 column of SDS-treated F60 (Fig. 1) showed a green fraction, termed the heavy (H) band, which eluted first, and a second green fraction, termed the light (L) band. The spectrum of each fraction in the presence of detergent is seen in Fig. 2. After extensive dialysis to remove the detergent, the absorption spectra of the H and L bands were recorded as shown in Fig. 3. The H band has a characteristic chlorophyll *a* absorption maximum at 676 nm, and the normal Soret region absorption. Additional absorption at 490–500 nm is a

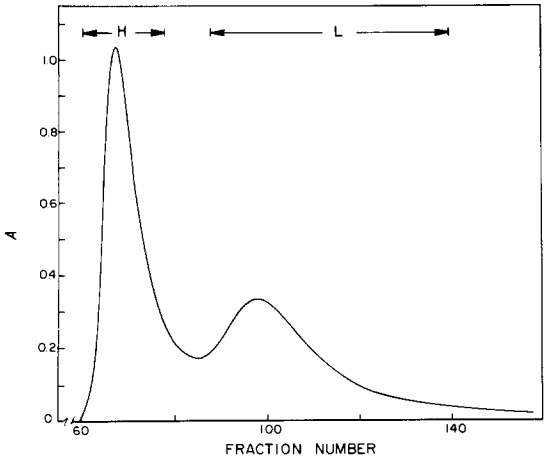

Fig. 1. Elution pattern of F60 from *P. luridum* on Sephacryl S-200 column with 0.05% SDS-Tris buffer (pH 8.0). Absorbance at 416 nm with a pathlength of 0.25 cm.

result of the presence of carotenoids. The L band absorption maximum is slightly lower at approx. 673 nm and the Soret absorption is more prominent at 418 than at 435 nm, probably as a result of some pheophytinization. There is an enhanced protein absorption at 280 nm in the L fraction. The ratio of 280 nm absorption to any of several peaks (340, 420, 435, 670) is much higher in the L fraction than in the H fraction.

A low-temperature emission peak of 728 nm was observed in the broken *P. luridum* cells in Tris buffer. In the presence of SDS, the low-temperature fluorescence emission spectra of the H and L bands resembled each other, with a major peak at 677 nm and a minor peak or shoulder at 720 nm; but after dialysis in Tris buffer the samples showed low-temperature fluorescence emission peaks at 715 and 686 nm for the H and L bands, respectively (Fig. 4).

Room-temperature emission spectra of four samples – F60 in the absence and presence of SDS, and the H and L bands immediately after Sephacryl S-200 chromatography – were examined. With the fluorescence yield of H (δ_{max}, 677 nm) defined as 1, the relative yields of F60 (λ_{max}, 678 nm), F60 in 0.05% SDS-Tris buffer (λ_{max}, 677 nm), and L (λ_{max}, 677 nm) were 1.01, 5.82, and 5.65, respectively; the samples had the same absorbance at the exciting wavelength (419 nm). The low fluorescence yields in the H band and in F60 without SDS suggested that the absorbed energy trapped inside the complex is greater than the emitted energy.

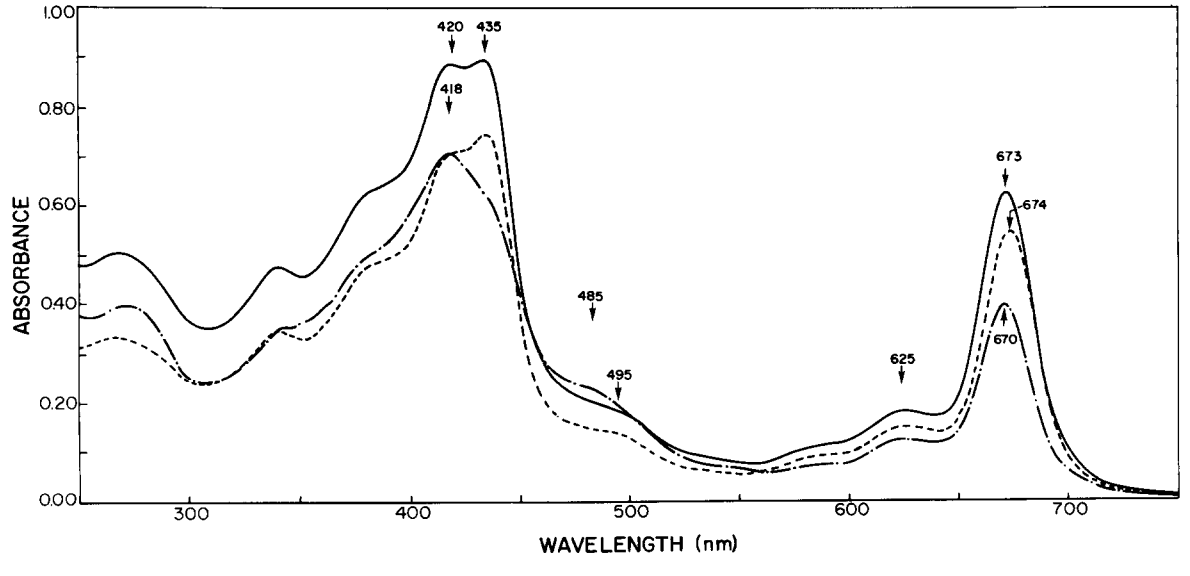

Fig. 2. Room-temperature absorption spectra of F60 from *P. luridum* after exposure to 0.05% SDS-Tris buffer, pH 8.0 (———), H (- - - - -) and L (·—·—·) fractions immediately after elution from Sephacryl S-200 column, in the presence of 0.05% SDS/Tris buffer (pH 8.0).

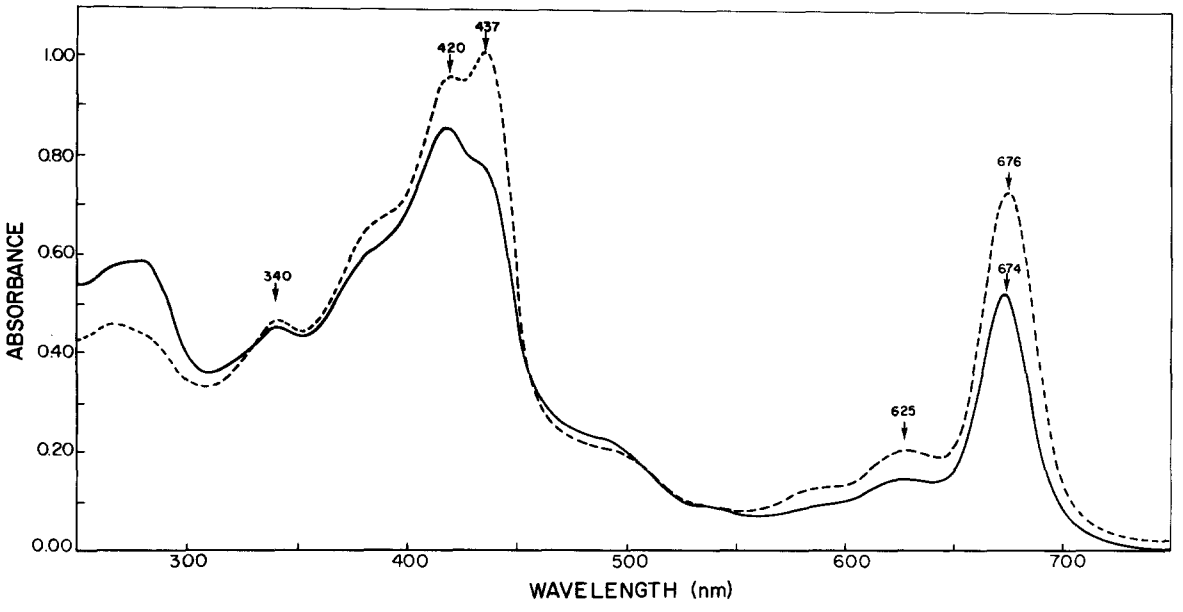

Fig. 3. Room-temperature absorption spectra of H (- - - - - -) and L (———) fractions. Spectra were taken in 50 mM Tris buffer after the detergent had been removed.

P-700 activity

The ferricyanide-ascorbate difference spectra of F60 and its two components differed greatly in the absence and presence of SDS (Fig. 5). In the absence of SDS F60 had a major trough at 700 nm, as did the H and L bands at 696 nm (Fig. 5a). In the presence of SDS-Tris buffer, F60 and the H fraction had two troughs as shown in Fig. 5b. Immediately after Sephacryl S-200 chromatography we observed one absorption minimum at 654 nm in the difference spectra of the L band (Fig. 5c). Quantitation of the chemically induced difference spectra in the H band yielded a Chl *a*/P-700 ratio ranging from 43 to 57. The ratio $\Delta A_{654} : \Delta A_{700}$ is greatest in the L fraction sample exposed to detergent and lowest in the H fraction sample.

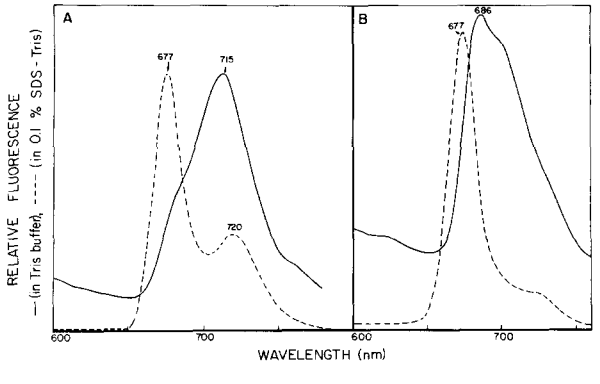

Fig. 4. Liquid-nitrogen temperature fluorescence emission spectra of the (A) H and (B) L fractions in 50% glycerol, 50 mM Tris buffer. ———, in Tris buffer; - - - - - -, in 0.1% SDS-Tris.

Polypeptide composition

The SDS-polyacrylamide gel electrophoresis analysis of unheated F-60 and H band exhibited eight Chl *a* bound proteins, accompanied by a low-molecular-weight (not more than 20 000) band (Fig. 6A). In the F60-unheated gels the bands (more than 20 kDa) are 264, 235, 214, 194, 176, 159, 124 and 90 kDa as compared with 229, 210, 194, 172, 150, 124, 90 and 72 kDa in H. The new band (72 kDa), present in the H fraction but not in F60, might result from the partial dissociation of the 90 kDa band. Only low-molecular-weight (13 000–16 000) polypeptides were seen in the L band on both gel systems. In addition to the 13–16 kDa polypeptides a single polypeptide (54 kDa) was observed in the denatured F60, and four

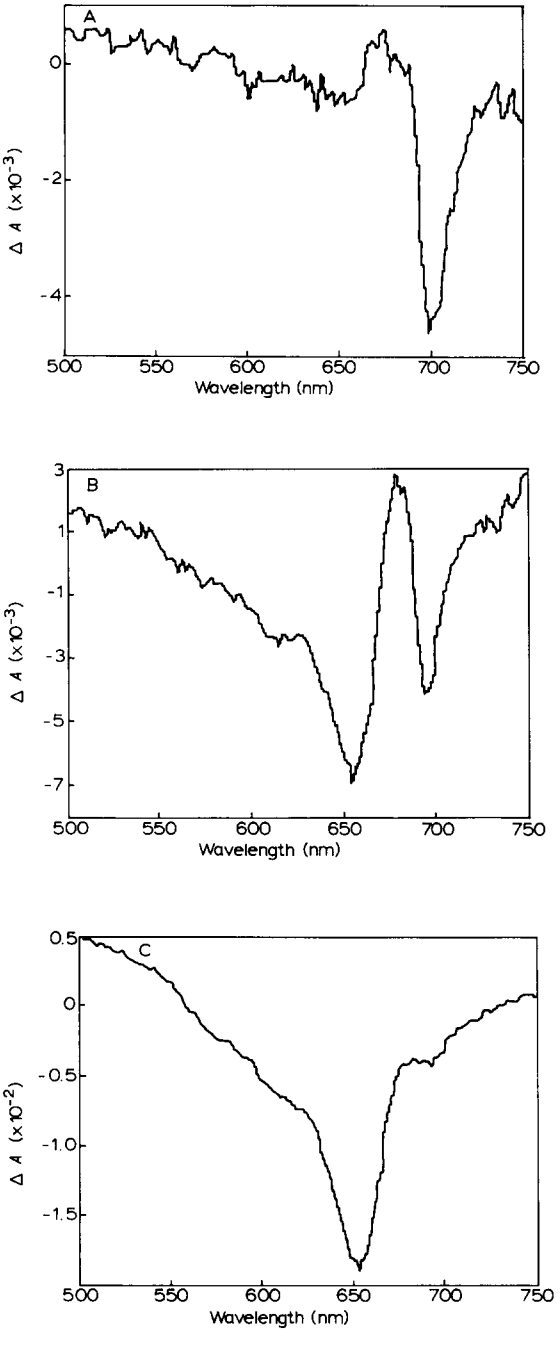

Fig. 5. Typical chemically induced difference spectra of F60 and L fraction. (a) F60 in Tris buffer, 5.5 μM in Chl *a*; note that H and L fraction in Tris buffer give similar spectra with slight shift of minimum to 696 nm. (b) F60 fraction in 0.05% SDS Tris buffer (pH 8.0), 42. μM in Chl *a*, note that fraction H in 0.05% SDS-Tris buffer (pH 8.0) has a similar spectrum except that the trough at 654 nm is quite small. (c) L fraction in 0.05% SDS-Tris buffer (pH 8.0), 3.94 μM in Chl *a*.

polypeptides (94, 54, 52 and 47 kDa) were observed in the H band.

Amino-acid composition

The amino-acid compositions of F60 and its two components are presented in Table I. Except for a significant difference in histidine content between the two components, the three samples had almost identical compositions. The amino acid analysis of the P-700 chlorophyll *a* protein from *P. luridum* previously reported by Thornber et al. [22] is included in Table I for comparison purposes.

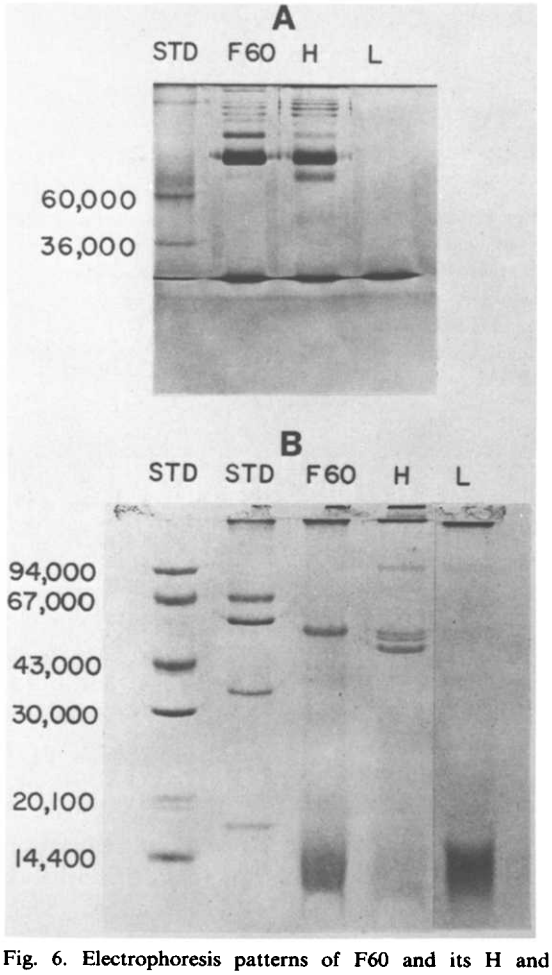

Fig. 6. Electrophoresis patterns of F60 and its H and L fractions. Analysis was by SDS-polyacrylamide gel electrophoresis at room temperature in gel containing (A) 7.5% acrylamide for the unheated sample or (B) 12% acrylamide for the heated sample (100°C, 2 min).

TABLE I

AMINO ACID COMPOSITIONS (mol%) OF F60 AND ITS H AND L FRACTIONS

Amino acid [a]	F60	H	L	P-700 [d]
Asp	8.2	9.0	8.1	8.8
Thr [b]	6.2	6.6	5.9	5.9
Ser [b]	6.6	6.9	8.2	5.9
Pro	5.0	4.6	4.1	4.7
Glu	6.8	6.6	7.5	6.5
Gly	10.5	11.1	11.4	10.0
Ala	10.4	10.4	9.6	10.0
Val	6.8	6.1	6.7	6.2
Cys [c]	1.7	2.2	1.7	0.9
Met	1.8	1.6	1.6	1.9
Ile	6.3	5.9	6.7	6.2
Leu	11.3	10.9	12.0	11.4
Phe	6.1	6.2	5.8	6.8
Tyr	2.7	2.8	2.2	2.7
Lys	2.9	2.7	3.1	2.9
His	2.8	3.0	1.1	5.9
Arg	3.9	3.4	4.2	3.6
Trp	–	–	–	1.1

[a] A total of nine analyses were made on the three hydrolysates (three each at 24, 48 and 72 h). The values shown are the averages of these nine determinations.

[b] Extrapolared from the 24 h, 48 h and 72 h acid hydrolyses.

[c] Determined as Cys after hydrolysis in the presence of me_2SO, according to Spencer and Wold [21].

[d] Amino acid analysis of P-700 Chlorophyll *a* protein from *P. luridum* reported by Thornber et al. [22].

Sedimentation velocity

The sedimentation coefficients of the H band in the absence and presence of detergent were extrapolated to zero Chl *a* concentration. Two species with sedimentation coefficients of 10.1 and 2.5 S were observed in the presence of SDS. After the detergent was removed by extensive dialysis, a single species of 14.7 S was observed. Two species reappeared when the detergent was added back to the solution. Apparently, there is an association between the 2.5 and 10.1 S species in the H band, and the reaction is reversible. In contrast, the L band contained a single species of 2.5 S with or without the detergent. The sedimentation coefficient for P-700-Chl *a* protein from *P. luridum* separated by sodium dodecyl sulfate extraction by Thornber et al. [22] was reported to be 9.1 S.

Discussion

This study was carried out to gain information about the properties of the components of F60. Two components, the H and L fractions, were separated on a Sephacryl S-200 column by SDS treatment. The separation is not well resolved when the ratio of SDS to Chl *a* (by weight) is lower than 6. The absorption spectrum of the H fraction in the presence of detergent (Fig. 2) is very similar to that for the entire F60 component in the presence of SDS. Dialysis of detergent overnight at 4°C in the dark results in spectral characteristics (Fig. 3) that compare favorably with the purest detergent prepared Photosystem-I reaction center preparation [10,32]. The appearance of additional absorption in the 490–500 nm region is a result of the presence of specific carotenoids in these preparations. Prolonged exposure of the H fraction to detergent and light does cause spectral shifts which are characteristic of pheophytinization (decrease in 435 nm absorption and increase in absorption at 505 and 538 nm).

The absorption in the 280 nm region in both the L and H fractions is most probably a contribution of principally the protein component of the chlorophyll proteins. The inability to stimulate fluorescence at longer wavelength characteristic of chlorophyll by irradiation at 280 nm is consistent with this suggestion. Examination of the ratio of 280 nm absorption to approx. 420 or approx. 670 nm absorption in H and L fractions clearly indicates a greater protein absorption in L than H. A simple interpretation of this is the greater unfolding to the protein and exposure of tyrosines and phenylalanines results in stronger absorption. Consistent with this opening of protein structure and accessibility of water is the consequence that the chlorophyll moiety will be more exposed to water, and therefore pheophytinization takes place. Magnesium porphoryins are known to be unstable in aqueous media, even in Tris buffer [29].

The isolation procedure also affected the Photosystem-I configuration, as indicated by the low-temperature fluorescence emission spectra. In Tris buffer the broken cells and the H band have emission peaks at 728 and 715 nm, respectively. A major 720 nm emission peak in F60 was reported previously [18]. Thus the 5 nm shift in the H band

is probably due to the disturbance of the Photosystem I configuration within the complex when the sample is exposed to SDS.

In the L band it seems unlikely that the 686 nm emission peak is PS II particles or light-harvesting Chl *a*-protein complexes. Newman and Sherman [11] have indicated that detergent-treated PS-II particles fluoresce at 683 nm at −196°C. Electron spin resonance studies, performed in collaboration with Dr. J. Warden, have demonstrated that the F60 fraction does not contain PS-II activity. With the evidence of the sedimentation coefficient (2.5 S), the absence of low temperature emission peak (approx. 720 nm) and no PS-II activity in F60 with or without the detergent, we have tentatively assigned the L band to the light-harvesting Chl *a*-protein.

Ogawa and Vernon [23] have isolated the P-700 fragment from *Anabaena variabilis,* with the Chl *a*/P-700 ratio of approx. 33. Klein and Vernon [24] have isolated a fraction from *Anabaena flas-aquae* with a ratio of 40. From the chemically induced difference spectra a Chl *a*/P-700 ratio ranging from 43 to 57 is calculated for the H band after SDS is removed. This value agrees well with the accepted Chl *a*/P-700 ratio of approx. 40 for detergent-extracted P-700 Chl *a*-protein complex. Similar experimental data with the L fraction are much less reproducible and indicate only a small amount of P-700 in this fraction (Chl *a*/P-700 > 300). Additional P-700 measurements have been performed in collaboration with Dr. J. Warden using the flash photolysis technique [30,31] and these results are consistent with minimal P-700 activity in the L fraction. In subsequent attempts at additional separation of the L band, one band has always been observed.

The appearance of a ΔA minimum at 654 nm in all samples exposed to detergent is most probably a result of irreversible oxidation of chlorophyll. This is a direct indication of the profound structural changes that occur on exposure to detergent. Assuming that the light-harvesting chlorophyll *a* is in vivo structurally different from the P-700 chlorophyll, and also assuming that the P-700 is in a more hydrophobic environment (hence the longer wavelength absorption) and therefore less accessible to solvent, several predictions can be made. (1) The preparation with the highest concentration of P-700 and lowest light-harvesting chlorophyll should have the lowest relative 654 nm contribution as indicated in the H band. (2) Conversely, the preparation with the highest light-harvesting chlorophyll concentration (the L band) should have the largest 654 nm contribution, and it does. The total F60 preparation has an intermediate relative concentration, as would be predicted. When the detergent is dialyzed from the H and L fractions, the intensity of the 654 nm peak is reduced. The Chl *a*/P-700 ratio is not affected in H by removing SDS. The accessibility of the P-700 to the chemical oxidants and reductants has apparently been maximized by the initial exposure to detergent, which probably uncouples the light harvesting and P-700 chlorophylls. Apparently, SDS causes a 4 nm shift in the difference spectra. After the detergent is added back to the H and L fractions, two peaks are observed: the peak position of 654 nm is time-dependent, while the peak position of 696 nm remains unchanged. Dietrich and Thornber [7] have observed a light-induced absorbance change at 697 nm in SDS-extracted P-700 Chl *a*-protein from *P. luridum,* and a small broad band at approx. 650 nm.

The polypeptide compositions of the two components of F60 are quite different. Both have low-molecular-weight (13–16 kDa) polypeptides and at this time it has not been possible to determine whether or not these low molecular weight peptides are the same in H and L. In the L fraction no higher molecular-weight species were detected, but eight such polypeptides (72–229 kDa) were seen in the unheated H band. When a sample of F60 was run in the same gel for comparison, the patterns of unheated F60 and H were almost identical except for one band of 264 kDa in F60 and one band of 72 kDa in the H band. In both cases the green band at 90 kDa was the major polypeptide.

Reiman and Thornber [27] isolated three Chl-protein complexes with molecular weights of 255 000, 118 000 and 58 000. Recently, Redlinger and Gantt [28] observed a green polypeptide at 95 kDa from the thylakoids. Reelectrophoresis of the green polypeptide after heating resulted in loss of the color and the appearance of a minor band at approx. 52 kDa and a 95 kDa polypeptide.

In our procedure, F60 was exposed to SDS

prior to SDS-polyacrylamide gel electrophoresis. Therefore, the alteration of the structure was kept to a minimum, and eight Chl *a*-bound polypeptides were detected in F60. In the denatured sample, one band of 53.7 kDa and a broad band of 13–16 kDa were found in F60 when the 12% gel was used. The broad band was resolved into four polypeptides of 18, 17, 16.5 and 16 kDa by a 10–15% gradient gel, as reported previously [18]. When a higher concentration of SDS (2%) was used to dissociate the F60 sample, three additional polypeptides of 94, 51.9 and 47.3 kDa were detected and appeared to be similar to those observed in the H band (data not included). The 94 kDa polypeptide is probably the dimer form of 47.3 kDa polypeptide. Therefore, the apparent appearance of new bands in denatured H over that found in denatured F60 (Fig. 6B) is actually representative of the differing sensitivity of H and F60 to SDS. Higher concentrations of SDS are required with F60 to produce the same effect as in H, and this is not surprising, since F60 contains lipid as well as other components. The aggregated material on the top of the resolving gel was caused by heating. Other investigators [25,26] have also noted that heating may cause aggregation.

The separation procedure used in this study separated a 2.5 S species, i.e., the L band, from F60. As mentioned above, we consider the L band to be the light-harvesting Chl *a*-protein complex. Thus the identity of the 2.5 S species in the H band requires further study. The 10.1 S species in the H band also needs further investigation for comparison with detergent-extracted CP I, which has a sedimentation coefficient of approx. 9 S [22]. The reversible association between two species (2.5 and 10.1 S) in the H band had never been reported. Based on the intermolecular interaction, the complicated polypeptide patterns in the H band may represent the various sizes of the oligomers. In addition, the histidine content of the H band is about 3-times that of the L band. The remaining amino-acid residues are almost identical between F60 and its two components. To understand the true relationship between these species in F60, the molecular weights of individual species must be determined.

In conclusion, the resolution of two components from F60 suggests that CP I [3,4], isolated by detergent solubilization, is similar to the H band in our preparation and the H band and F60 are similar in amino acid composition, polypeptide patterns, and spectroscopic properties. The H and L components also have similar amino acid compositions.

The presence of the 10.1 S species, the 715 nm emission peak, the chemicallly induced 696 nm peak, and the low fluorescence yield indicate that P-700 is located principally in the H band.

Separated 2.5 and 10.1 S species from the H band self-aggregate, but 2.5 S from the L band does not. Further characterization of the 2.5 S species may show whether the 2.5 S species in the H band is identical to the L band.

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